

Comparative Studies on the Ultrastructure of Nucleoli in Human Lymphosarcoma Cells and Leukemic Lymphocytes¹

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SUMMARY

The ultrastructural morphology of nucleoli was investigated in lymphosarcoma cells and leukemic lymphocytes of patients who were not treated with antitumor or anti-leukemic therapy. The nucleoli in lymphosarcoma cells were compact, contained well-defined nucleolonemas, or were ring-shaped; occasionally, transitional forms between these types of nucleoli were observed. The ultrastructure of the ring-shaped nucleoli present in differentiated lymphosarcoma cells was similar to that of mature leukemic lymphocytes. These nucleoli were characterized by a peripheral ribonucleo-protein shell surrounding a central core, adjacent to which there were chromatin clusters. A decrease of the granular components was noted in the ring-shaped nucleoli of both differentiated lymphosarcoma cells and mature leukemic lymphocytes, as compared with the compact nucleoli or nucleoli with nucleolonemas of the less differentiated lymphosarcoma cells or immature leukemic lymphocytes. The decrease of granular components in the ring-shaped nucleoli may reflect a low rate of the formation of granular nucleolar ribosomal precursors. In addition, small microspherules were noted in nucleoli of some lymphosarcoma cells.

INTRODUCTION

Nucleoli in rapidly growing and proliferating cells are usually compact or they are composed of more or less developed nucleolonemas (2). On the other hand, nucleoli in some mature or resting cells can be characterized by their ring-shaped appearance. Particular attention has been directed in this laboratory to ring-shaped nucleoli which are characterized by the presence of ribonucleoprotein structures in their periphery which form a shell surrounding a central light core. Such nucleoli were observed in some insect cells (29), kangaroo rat hepatocytes (6), human mature lymphocytes, plasmacytes, and monocytes, as well as in some immature cells of the granulocytic series (13, 20-22). Similar nucleoli were also produced in rat liver cells or

various cultured cells by actinomycin D or chromomycin A₃ (11, 12, 23). In all these cell types, the nucleolar shape seems to reflect a low rate of nucleolar RNA synthesis.

Although the ultrastructural morphology of undifferentiated and well-differentiated cells of lymphosarcomas (poorly and well-differentiated malignant lymphomas) is very similar to leukemic lymphocytes in various stages of their development and maturation (4, 5), the present studies are the first to compare the ultrastructure of the various types of nucleoli in lymphosarcoma cells to those of leukemic lymphocytes (8) and to show the presence of ring-shaped nucleoli in lymphosarcoma cells. The distribution of granular and fibrillar components of ring-shaped nucleoli of these cells also was analyzed.

The ultrastructure of ring-shaped nucleoli in lymphosarcoma cells did not differ from that in leukemic lymphocytes. There was a decrease of granular components noted in ring-shaped nucleoli of both leukemic lymphocytes and differentiated lymphosarcoma cells. In addition, microspherules were observed in some nucleoli of lymphosarcoma cells.

MATERIALS AND METHODS

Electron microscopic studies were carried out on samples of lymph nodes of 5 patients with lymphosarcomas, 1 with a poorly differentiated malignant lymphoma and 4 with well-differentiated malignant lymphomas. Samples were also studied from a lymph node and bone marrows of 4 patients with chronic lymphocytic leukemias. The peripheral blood of patients with lymphosarcomas did not contain leukemic cells and the differential count of leukocytes appeared to be normal. The peripheral blood of patients with chronic lymphocytic leukemia showed typical changes characteristic for this disease, *i.e.*, the total number of leukocytes ranged from 51,000 to 65,000/cu mm; the majority were mature lymphocytes.

The samples for electron microscopy were fixed in glutaraldehyde (15) and postfixed in osmium tetroxide (10). After dehydration in a graded series of ethanol containing uranyl acetate, the specimens were embedded in Epon-Araldite (9). Ultrathin sections cut with an LKB Ultratome were stained with uranyl acetate, followed by lead citrate (27, 28), and observed with a Philips 200 electron microscope. The ratios of areas containing granular components of the nucleolus

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were determined on the electron micrographs (X 300,000 to 60,000) of nucleolar sections with a planimeter OTT, Burrell Corp., Pittsburgh, Pa., (24). Approximately 10 to 15 sections were investigated from each group of (a) ring-shaped nucleoli of differentiated lymphosarcoma cells, (b) ring-shaped nucleoli of mature leukemic lymphocytes, (c) compact nucleoli and nucleoli with nucleolonemas of lymphosarcoma cells, and (d) compact nucleoli and nucleoli with nucleolonemas of leukemic lymphoblasts.

RESULTS

Lymphosarcomas (Well- and Poorly Differentiated Malignant Lymphomas)

Compact Nucleoli. These (Fig. 1) and nucleoli with nucleolonemas (Fig. 2) were usually present in less-differentiated cells.² In comparison with ring-shaped nucleoli, the granular components appeared to be more prominent, since their number, as well as the areas containing them, were large (Table 1).

Table 1
Percentage of the total nucleolar area containing granular components

	Compact nucleoli and nucleoli with nucleolonemas	Ring-shaped nucleoli
Lymphosarcomas (29) ^a	79 ± 11	54 ± 14
Lymphocytic leukemias (22)	82 ± 5	49 ± 8

^aNo. of nucleoli studied.

Ring-shaped Nucleoli. These (Figs. 3 to 5) were usually observed in differentiated cells (Fig. 4) which resembled mature lymphocytes. However, these nucleoli were also noted in a few less-differentiated cells, as well as in some triangular or elongated cells, the classification of which was not possible (Figs. 3 and 6).

The ultrastructural morphology of ring-shaped nucleoli was very characteristic. The peripheral nucleolar shell was composed of ribonucleoprotein components. The fibrillar com-

ponents were usually present around the central light core (Fig. 4). The number of granular components seemed to be reduced (Fig. 4) and areas containing them appeared to be smaller (Table 1), as compared with compact nucleoli or nucleoli with nucleolonemas. The central light cores of ring-shaped nucleoli were composed of fine filaments and chromatin clusters³ were usually found at their periphery (Fig. 4). In some sections of ring-shaped nucleoli, the peripheral ribonucleoprotein ring was open and chromatin penetrated in this region to the central light area (Fig. 5).

Transitional forms between ring-shaped nucleoli and nucleoli with nucleolonemas or compact nucleoli were also observed (Figs. 3 and 7). Nucleoli that lacked nucleolonemas but contained light areas in the centers probably represented transitional forms between compact nucleoli and ring-shaped nucleoli (Fig. 3). The transitional forms between ring-shaped nucleoli and nucleoli containing nucleolonemas were characterized by a central light core and by the presence of nucleolonemas in the nucleolar peripheral shell (Fig. 7). In contrast to typical ring-shaped nucleoli, granular components seemed to be more prominent in such nucleoli.

In addition, "microspherules" 350 to 600 Å in diameter were observed in the nucleoli of lymphosarcoma cells of 2 patients (Figs. 3, 7 to 9). These structures were present in nucleolar light areas or were surrounded by small light halos (25, 26). Some of the microspherules seemed to be linked to nucleolonemas or surrounding nucleolar components; although these structures are not completely spherical, no long strands were seen in any plane studied and probably they were not parts of longer channels. At higher magnifications they contained filaments which were similar to fibrillar components and their density seemed to be lower than that of chromatin fibrils (Fig. 8).

Chronic Lymphocytic Leukemia

Ring-shaped nucleoli were present predominantly in mature lymphocytes⁴ (Fig. 10), as observed in previous studies (21, 22). They were characterized by the presence of ribonucleoprotein components in their peripheral part, which formed a ring surrounding a central light area (Fig. 11). In the central light area, the fine filaments and chromatin clusters present were usually adjacent to its periphery (Fig. 11). Chromatin structures penetrated through the peripheral nucleolar ribonucleoprotein shell to the central light area (Figs. 10 and 11). Areas containing fibrillar components were more prominent in ring-shaped nucleoli (Fig. 11), as compared with

² Less-differentiated or undifferentiated lymphosarcoma cells represent a population of immature cells which can be classified as lymphoblasts, hemocytoblasts, or reticulum cells (4). These cells are characterized by more or less irregularly shaped nuclei with large hypertrophied nucleoli and abundant cytoplasm with large mitochondria and numerous ribosomes, which can form rosettes (especially in hemocytoblasts). Differentiated lymphosarcoma cells similar to lymphocytes (4) are characterized by dense nuclei with large chromatin clusters and small nucleoli. Deep cytoplasmic nuclear invaginations or deep unilateral clefts of the nuclear membrane are frequent. The cytoplasm containing ribosomes and the few mitochondria usually form a small rim around the nucleus in most cells.

³ The identification of DNA in chromatin and other nucleolar substructures was made in earlier studies from this laboratory by means of selective enzymatic degradation (26).

⁴ The ultrastructure of mature leukemic lymphocytes is characterized by a nucleus that contains large chromatin clumps and small nucleoli. A small cytoplasmic mass forms a rim around the nucleus; it contains ribosomes and few mitochondria. The chromatin of leukemic lymphoblasts is less clumped and the nucleoli are larger than those of mature lymphocytes. The abundant cytoplasm of lymphoblasts is filled with ribosomes and contains large mitochondria.

compact nucleoli or nucleoli with nucleolonemas, which were observed in immature cells (Fig. 12). On the other hand, the number of granular components, as well as the areas containing them, seemed to be reduced in ring-shaped nucleoli (Table 1) in comparison with compact nucleoli or nucleoli with nucleolonemas of immature cell forms.

DISCUSSION

The present study has shown that lymphosarcoma cells contain a variety of types of nucleoli, including compact nucleoli, nucleoli with well-defined nucleolonemas, and ring-shaped nucleoli. The present observations also indicated that ring-shaped nucleoli of differentiated lymphosarcoma cells, as well as mature leukemic lymphocytes, are characterized by a decrease of ribonucleoprotein particles, as compared with compact nucleoli or nucleoli with nucleolonemas found in less-differentiated lymphosarcoma cells or leukemic lymphoblasts. Thus, the presence of ring-shaped nucleoli in many lymphosarcoma cells suggests that in these cells the synthesis of nucleolar ribonucleoprotein particles representing ribosomal precursors (3) is repressed, as it is in mature normal or leukemic lymphocytes. A similar decrease of nucleolar ribonucleoprotein particles was produced by the inhibition of RNA synthesis with actinomycin D. The low rate of ribosomal RNA synthesis in mature leukemic lymphocytes is well known (3, 14, 24). The finding of ring-shaped nucleoli in differentiated lymphosarcoma cells also supports the concept that in many cells of these neoplasms the biosynthetic reactions leading to the cell growth and division operate at low rates. This finding is consistent with the concept that such cells are resting in G_0 phase or a prolonged interphase (1, 16, 17, 19). On the other hand, the presence of large compact nucleoli rich in nucleolar ribonucleoprotein particles in less-differentiated cells indicates that these cells are rapidly synthesizing ribosomal precursors and represent cells that are very active in biosynthetic reactions.

The "spots" (microspherules) in "spotted nucleoli" (2-4) of lymphosarcoma cells are of interest because similar structures have also been observed in the spotted nucleoli of the Reed-Sternberg cells of Hodgkin's disease (2, 7). However, the structures in Reed-Sternberg cells were larger and less regular in shape. The ultrastructure of the spots (microspherules) in spotted nucleoli of lymphosarcoma cells resembles that of the RNA-containing microspherules in nucleoli of actinomycin D-treated tumor cells or hepatocytes pretreated with thioacetamide (18, 25). On the other hand, the microspherules in spotted nucleoli of lymphosarcoma cells seem to be different from large and dense polymorphous granules in spotted nucleoli of various cells infected with viruses (2, 3). Like the microspherules in tumor cells treated with actinomycin D, the microspherules in lymphosarcoma cells contained fine fibrils similar to nucleolar fibrillar components; they were also surrounded by light halos. However, the microspherules in lymphosarcoma cells were small and their fibrillar structures seem to be less condensed and coiled.

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Figs. 1–7 and 9–12. The measured line is 1 μ .

Fig. 1. Compact nucleolus with small light areas. Such nucleoli are characterized by large quantities of granular components. *Pointer*, small chromatin clusters in light area. X 30,500.

Fig. 2. Nucleolus with prominent nucleolonemas containing mainly dense granular elements. *Pointer*, intranucleolar chromatin. X 51,500.

Fig. 3. Compact nucleolus with large light area (*L*) and ring-shaped nucleolus with small spots (*white pointers*). X 37,000.

Fig. 4. Ring-shaped nucleolus (*arrow*) in a differentiated lymphosarcoma cell, showing a decrease of granular components. *L*, light central core; *F*, fibrillar components; *pointer*, clusters of intranucleolar chromatin. X 46,200.

Fig. 5. Ring-shaped nucleolus with an opening containing chromatin in its peripheral ring (*pointer*). X 37,500.

Fig. 6. Elongated cell in a lymphosarcoma with a ring-shaped nucleolus (*arrow*). X 23,000.

Fig. 7. Transitional form between ring-shaped nucleolus and nucleolus with nucleolonemas containing small spots (*pointers*). X 59,000.

Fig. 8. High magnification of nucleolus in Fig. 7 with small spots composed of fine fibrils (*pointers*). *Arrow*, intranucleolar chromatin. *Black line*, 0.1 μ . X 110,000.

Fig. 9. Compact nucleolus with small spots (*pointers*). X 46,000.

Fig. 10. Ring-shaped nucleolus in a mature leukemic lymphocyte (*arrow*). X 25,000.

Fig. 11. Ring-shaped nucleolus (*arrow*) of a mature lymphocyte. *Pointer*, fibrillar components; *small arrows*, clusters of intranucleolar chromatin. X 34,000.

Fig. 12. Nucleolus of a leukemic lymphoblast with numerous granular elements. X 29,000.





